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(54) Title: NEUROPEPTIDE Y ANTAGONISTS

(57) Abstract

The present invention relates to peptides which show improved Neuropeptide Y antagonism, to pharmaceutical compositions containing such peptides and to their use in animal and human medicine.

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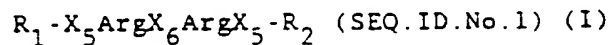
NEUROPEPTIDE Y ANTAGONISTS.

The present invention relates to peptides which show improved Neuropeptide Y antagonism, to pharmaceutical compositions containing such peptides and to their use in animal and human medicine.

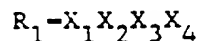
Neuropeptide Y (NPY) is a 36 residue amidated peptide first isolated from brain tissue in 1982 (Tatemoto K., Carlquist M. and Mutt V., *Nature* 296; 659-660, 1982). The peptide is widely distributed throughout the mammalian central nervous system and the peripheral sympathetic nervous system; in the latter it is colocalised with norepinephrine. Peripheral administration of NPY induces vasoconstriction in many vascular beds and also potentiates the vasoconstriction induced by norepinephrine, and other vasoactive substances, in vessels where NPY does not have a direct effect. NPY functions in the brain as an appetite stimulant and promotes release of prolactin growth hormone and leutinizing hormone. NPY is known to elicit its effect by binding to receptors located in the central and peripheral nervous system. EPO 355 794 and DE 38 11 193 describe derivatives of NPY which may bind to such NPY receptors and act as antagonists of NPY activity.

A class of peptides has now been found which are effective NPY antagonists. These antagonists suppress the action of endogenous NPY and are therefore useful in cardiovascular disorders, hypertension and hypertensive crisis, vasospasm, cerebral or coronary vasospasm (eg. stroke), eating disorders, depression and also in glaucoma. Such compounds are particularly suitable for use in combination with angiotensin converting enzyme (ACE) inhibitors and calcium antagonists.

The present invention provides a peptide of formula (I), or a multimer thereof or a salt thereof:



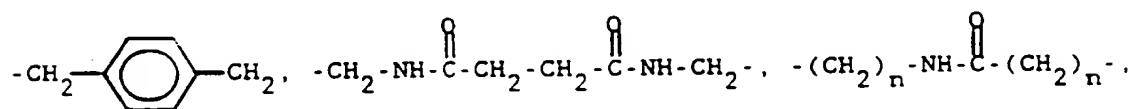
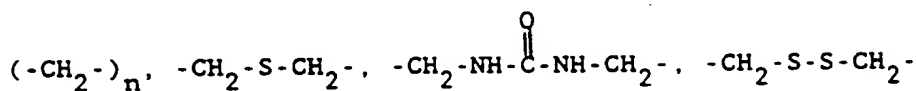
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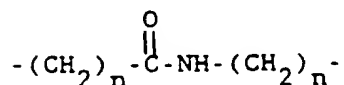
in which X_1 -Ile or TyrIle, or desNH₂TyrIle
 X_2 -Asn, Asp, Cys, Dpr, Glu or Gly
 X_3 -Leu, Pro or 3,4-DehydroPro
 X_4 -Aib, Asp, Cys, Dpr, Glu, Gly, Ile, Orn, Tyr or O-MethylThr
 X_5 -Phe, Tyr or [O-(2,6-Dichlorobenzyl)Tyr]
 X_6 -Phe or Leu

where R_2 --(CH₂)_nCH₃, --(CH₂)_nNH₂ or $\text{--}\overset{\text{O}}{\parallel}\text{C--CH}_3$ in which n=0-4.

A multimer according to the present invention includes a dimer or trimer. Such normally occur when peptides containing Gly residues are bridged at the alpha position by a group selected from the following:



and



in which n is as defined herein. Alternatively, such multimers may also occur when peptides are lactam bridged. Such multimers can contain one or more such bridges, preferably two.

It is preferred that a peptide of the invention is used in the form of a dimer.

A salt of a peptide or a multimer of the invention is also included within the scope of the present invention. Such a salt is prepared according to any of the methods well known in the art.

A pharmaceutically acceptable salt of a peptide or a multimer of the invention is also included within the scope of the present invention. Suitable pharmaceutically acceptable salts include acid addition salts when the peptide is sufficiently basic i.e., contains one or more basic residues.

A suitable pharmaceutically acceptable acid addition salt of a peptide of the invention may be formed with an inorganic acid, for example hydrochloric acid, hydrobromic acid, sulphuric acid or phosphoric acid, or with an organic acid, for example acetic acid, citric acid, maleic acid, fumaric acid, succinic acid, tartaric acid or trifluoroacetic acid.

Preferred examples of X_2 include Cys and Glu.

Preferred examples of X_3 include Pro.

Preferred examples of X_4 include Cys Dpr and Orn.

Preferred examples of X_5 include Tyr.

Preferred examples of X_6 include Leu.

Preferred examples of R_2 include $-(CH_2)_nNH_2$ in particular wherein n is 0.

A preferred sub-class of a peptide of formula (I), or a multimer thereof, or a salt thereof, is that wherein X_5 is Tyr, X_6 is Leu, and R_1 and R_2 have the meaning described above.

The following abbreviations for amino acid residues are used throughout:

Aib for 2-methyl alanine, Arg for arginine, Asn for asparagine, Asp for aspartic acid, Cys for cysteine, Dpr for diaminopropionic acid, Glu for glutamic acid, Gly for glycine, Ile for isoleucine, Leu for leucine, Orn for ornithine, Phe for phenylalanine, Pro for proline, Thr for threonine and Tyr for tyrosine.

The present invention relates particularly to the following peptides:

IleGluProOrnTyrArgLeuArgTyr-NH₂,Cyclic(2,4'),(2',4)-Diamide
(SEQ.ID.No:2)

IleAspProOrnTyrArgLeuArgTyr-NH₂,Cyclic(2,4'),(2',4)-Diamide
(SEQ.ID.No:3)

IleGluProDprTyrArgLeuArgTyr-NH₂,Cyclic(2,4'),(2',4)-Diamide
(SEQ.ID.No:4)

IleAspProDprTyrArgLeuArgTyr-NH₂,Cyclic(2,4'),(2',4)-Diamide
(SEQ.ID.No:7)

IleDprProAspTyrArgLeuArgTyr-NH₂,Cyclic(2,4'),(2',4)-Diamide
(SEQ.ID.No:5)

and

IleCysProCysTyrArgLeuArgTyr-NH₂,Cyclic (2,2'),(4,4')-Disulfide Dimer,
(SEQ.ID.No:6)

IleCysProCysTyrArgLeuArgTyr-NH₂,Cyclic (2,4'),(4,2')-Disulfide Dimer
(SEQ.ID.No:6)

or a pharmaceutically acceptable salt thereof.

The invention relates most particularly to the following peptides:

IleGluProOrnTyrArgLeuArgTyr-NH₂,Cyclic(2,4'),(2',4)-Diamide
(SEQ.ID.No:2)

IleAspProOrnTyrArgLeuArgTyr-NH₂,Cyclic(2,4'),(2',4)-Diamide
(Seq.ID.No:3)

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IleGluProDprTyrArgLeuArgTyr-NH₂,Cyclic(2,4'),(2',4)-Diamide
(SEQ.ID.No:4)

IleAspProDprTyrArgLeuArgTyr-NH₂,Cyclic(2,4'),(2',4)-Diamide
(SEQ.ID.No:7)

and

IleDprProAspTyrArgLeuArgTyr-NH₂,Cyclic(2,4')(2',4)-Diamide
(SEQ.ID.No:5)

or a pharmaceutically acceptable salt thereof.

According to the present invention there is also provided a method for the treatment of cardiovascular disorders, hypertension, hypertensive crisis, vasospasm, cerebral or coronary vasospasm (eg. stroke), eating disorders, depression and also glaucoma, which comprises administering to a mammal e.g. human, a therapeutically effective, non-toxic amount of a peptide or a multimer or a salt according to the present invention. There is also provided the use of a peptide or a multimer or a salt according to the present invention in the manufacture of a medicament for the treatment of any of the above-mentioned conditions.

Peptides of formula (I) of the present invention wherein X₃ is Leu also exhibit agonistic properties. These agonists can mimick or enhance the actions of endogenous NPY and are therefore useful in treating conditions requiring activation of NPY receptors, for example as vasoconstrictors. More particularly they are useful in the treatment of hypotension, vasodilation, septic shock, rhynitis, cystic fibrosis, anxiety and in the protection against nephro-toxicity.

According to a further feature of the invention there is provided a pharmaceutical composition which comprises a peptide of the present invention, or a multimer thereof, or a pharmaceutically acceptable salt

thereof, in association with a pharmaceutically acceptable diluent or carrier.

The composition may be in a form suitable for oral use, for example a tablet, capsule, aqueous or oily solution, suspension or emulsion; for nasal use, for example, a snuff, nasal spray or nasal drops; for optical use, for example, eye drops; for vaginal or rectal use, for example a suppository; for administration by inhalation, for example as a finely divided powder or a liquid aerosol; for sub-lingual or buccal use, for example a tablet or capsule; or for parenteral use (including intravenous, subcutaneous, intramuscular, intravascular or infusion), for example a sterile aqueous or oily solution or suspension.

In general the above compositions may be prepared in a conventional manner using conventional excipients. However, in the case of a composition for oral administration, it may be convenient for the composition to include a coating to protect the peptide active ingredient from the degradative actions of enzymes in the stomach.

An oral composition of the invention is, in unit dosage, for example a tablet or capsule which contains from 2.5 to 500mg, and preferably 1 to 200mg, of peptide in each unit dose, or one suitable for parenteral administration which contains from 0.5 to 100mg of peptide per ml, and preferably 1 to 10mg of peptide per ml of solution.

A parenteral composition is preferably a solution in isotonic saline or isotonic dextrose buffered if necessary to a pH of 5 to 9. Alternatively, the parenteral composition may be one designed for slow release in which case the amount of polypeptide per unit dose is in general greater than that required when a conventional injectable formulation is used. A preferred slow release formulation is, for example, a continuous release formulation, for example a formulation of the type described in US4767628 and US5004602 which are incorporated herein in their entirety. A preferred slow release parenteral formulation contains from 10 to 100 mg of polypeptide per unit dose. Another preferred slow release formulation is a

micro-encapsulated polypeptide using a biodegradable biocompatible copolymer.

These preparations are preferably administered intravenously, although administration may also be effected by means of subcutaneous, intramuscular, or intradermal injection.

Medicaments suitable for transdermal administration may take the form of an optionally buffered aqueous solution of a compound of the general formula, or a pharmaceutically acceptable salt thereof and may be delivered by passive diffusion or by electrically-assisted transport, for example, iontophoresis (see, for example, Pharmaceutical Research 3(6), 318 (1986)).

The composition of the invention will normally be administered such that a daily oral dose will be from 0.1mg/kg, to 50mg/kg of bodyweight and most preferably from 0.5mg/kg to 10mg/kg and a daily parenteral dose, will be from 20 micrograms/kg to 10mg/kg more preferably from 50 micrograms/kg to 2mg/kg.

The peptides of the invention may be prepared by any process well known in the art of peptide chemistry to be applicable to the synthesis of analogous compounds. Thus, for example, a polypeptide of the invention may be obtained by procedures analogous to those disclosed in "Solid Phase Peptide Synthesis" by Stewart and Young (published by the Pierce Chemical Company, Illinois, 1984), "Principles of Peptide Synthesis" (published by Springer-Verlag, Berlin, 1984), "Practice of Peptide Synthesis" (published by Springer-Verlag, Berlin, 1984), and "The Synthesis of a Tetrapeptide (J. Am. Chem. Soc., 83 2149(1963)).

The multimers of the invention may be prepared by the methods described hereinafter or by any process well known in the art of peptide chemistry.

The salts of the invention may be prepared by any process well known in the art.

PEPTIDE SYNTHESIS AND PURIFICATION

The peptides were synthesized using an improved version of the solid phase method described by R.B. Merrifield, "Solid Phase Peptide Synthesis I. The Synthesis of a Tetrapeptide", J.Am.Chem., 83, 2149 (1963), using an Applied Biosystems Model 430A peptide synthesizer.

The MBHA resin was obtained from Advanced Chemtech of Louisville, Ky. The BOP reagent was received from Richelieu Biotechnologies in St-Hyacinthe, Quebec, Canada. The majority of the natural Boc- and Fmoc-protected amino acids utilized were obtained from the Bachem Chemical Co. of Torrance, CA, including Boc-Cys(4MeBzl), Boc-Phe, Boc-His(CBZ), Boc-Ile.1/2H₂O, Boc-Leu.H₂O, Boc-Asn(Xan), Boc-Nle, Boc-Pro, Boc-Arg(Tos), Boc-Trp, Boc-Tyr(2-Br-CBZ), Fmoc-Cys(Trt), Fmoc-Ile, Fmoc-Leu, Fmoc-Asn(Trt), Fmoc-Pro, Fmoc-Arg(PMC), Fmoc-Tyr(Ot-butyl), and Fmoc-Tyr(O-2,6-dichlorobenzyl). Bachem of California also supplied Boc-6-aminocaproic acid (Aha) and Boc-3,4-dehydropyrroline. The O-methylhomoserine (MeOThr) was supplied by Biohellen S.A. of Greece. The Boc-aminoisobutyric acid (Aib) and parahydroxyphenylpropionic acid [(de-NH₂)Y] was obtained from the Fluka Chemical Corp. of Ronkonkoma, NY. Boc-benzothienylalanine (BzThiAla) was obtained from SyntheTech of Albany, OR. The Aldrich Chemical Co. of Milwaukee, WI supplied 1-methylimidazole, phenylpropionic acid [(de-NH₂)F], and 3,4-dichlorophenylacetic acid. Boc-Asp(OFm) and 1-Naphthyl-3-alanine were obtained from Bachem Bioscience Inc. of Philadelphia, PA.

Method A: Peptide Synthesis Using t-Boc Chemistry

Most peptides were synthesized by the Boc-protection procedure as follows:-

Boc-protected amino acids were coupled to the MBHA (methyl benzhydryl) resin (Advanced Chem.Tech, Louisville, Kentucky Catalog 1990-91 p129, SA5016) using a programme to suit a BOP coupling procedure, as described by Dung Le-Nguyen, Annie Heik, and Bertrand Castro, J.Chem.Soc., Perkins Trans. 1, 1914 (1987). The coupling protocol involved dissolving 1 mMole

of Boc-protected amino acid, 1 mMole BOP, and 1 mL of 1 M 1-methylimidazole in 7 mL of DMF. The mixture was added to 0.5 mMoles of resin, mixed for 1 hour, and filtered. This procedure was performed automatically in an Applied Biosystems 430A peptide synthesizer with the Boc-protected amino acid and BOP filled in the amino acid cartridge using a short programme routine to perform the above coupling steps. Afterwards, a series of DMF and CH_2Cl_2 washes and TFA deprotection steps was performed in an Applied Biosystems model 430A peptide Synthesizer using programmes supplied by the manufacturer (Applied Biosystems, Foster City, CA) and this procedure was automatically repeated for each desired amino acid until the desired sequence is assembled in the synthesizer.

After the peptide was assembled on the resin, it was deblocked and cleaved from the resin with liquid HF containing 10% anisole, in a variation of the method described by S. Sakakibara, et al., in Bull.Chem.Soc.Jap., 40, 2164(1967). The peptide and resin were next washed with ethyl acetate and the peptide was extracted from the resin with an aqueous 1-10% acetic acid solution. The peptide solution was then lyophilized to obtain the dry, solid peptide.

Peptides containing disulfide bridges were prepared as described in the Examples.

Method B: Peptide Synthesis Using Fmoc Chemistry

Several peptides contained functionalities that were labile against strong acid and were synthesized by the Fmoc-protection procedure. The peptides were assembled by an automated method on a Milligen 9050 peptide synthesizer using BOP as the coupling reagent. A 4-(2',4'-dimethoxyphenyl-FMOC-aminomethyl)-phenoxy resin ("Rink resin") (Bachem Torrance, California, Catalogue 1991-92 p136 RMIS70), (2.2g, 0.198 mM) was combined with 3.2 g of acid washed glass beads (Sigma, 250-212 micron size) and loaded into the flow-through reaction vessel of the peptide synthesizer. The appropriate side chain protected FMOC amino acids were placed into individual cartridges in 0.8 mmole allotments. BOP Reagent

(354 mg, 0.8 mmol) and 1-Hydroxy-benzotriazole (122 mg, 0.9 mmol) were added to each cartridge before sealing. The automated synthesis required about 20 h. The peptides were cleaved from the resin using a standard trifluoroacetic acid method. The resin/glass bead mixture was introduced into a plastic screw-top Sarstedt tube (50 mL capacity). To it was added 10 mL of the following solution: 9 mL of TFA, 0.5 mL of thioanisole, 0.3 mL of ethanedithiol, and 0.2 mL of anisole. This cleavage reaction mixture was allowed to stand for 4 h. The resin and glass beads were filtered and the TFA was removed from the filtrate in vacuo. Diethyl ether was poured on the residue and the peptide precipitated, was filtered, washed with ether and dried. The crude peptide was purified by the standard preparative HPLC chromatography procedure.

The peptides were then purified by reverse-phase liquid chromatography using a Waters Pre-Pak (Delta-Pak C₁₈) column on a Waters Delta Prep 4000 system equipped with a Waters 484 ultraviolet detector. Purification was achieved by equilibrating the column with 0.1% TFA in water and developing with a linear gradient of acetonitrile from 1-40% in 20 minutes at a flow rate of 20 mL/min at 220 nm. Samples were collected manually and checked for purity on a Waters analytical HPLC system (including a 600E pump and system controller, a 494 photodiode array detector, and a 712 WISP autosampler) utilizing a Waters Radial-Pak (Delta-Pak C₁₈) column. A flow rate of 2.5 mL/min was employed using a 0.1% TFA/acetonitrile gradient from 10-60% ACN in 10 minutes at 200 nm.

Method C: Dimeric Nonapeptides (amidebridged)

Synthesis of protected 2,3-diaminopropionic acid:

(S)-2-((tert-butoxycarbonyl)amino)-3-((9H-Fluoren-9-ylmethoxy)carbonyl)amino)propionic acid: A solution of 10 g (42 mmol) of N- α -CBZ-diaminopropionic acid (Fluka, Catalogue 1990-91 p1368 No. 96085) in 75 mL of 1,4-dioxane was treated with 75 mL of 1N aqueous sodium hydroxide, followed by 9.1 g (35 mmol) of 9-fluorenylmethyl chloroformate (Aldrich, Catalogue 1992-93 p620 No. 16,051-2) and 5 g (15 mmol) of N-(9-fluorenylmethoxycarbonyloxy)succinimide (Aldrich, Catalogue 1992-93

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p620 No. 28,950-7). The resulting mixture was stirred at 25°C for 4 h and then acidified to pH 2 with concentrated aqueous hydrochloric acid and extracted with ether. Drying over magnesium sulfate and evaporation of the volatiles afforded a white foam, which was redissolved in 150 mL of 30% hydrogen bromide in acetic acid. After stirring for 1 h at 25°C, the volatiles were removed in vacuo and residual acetic acid was co-evaporated three times with toluene. Trituration with ether afforded a beige solid which was slurried in 1,4-dioxane (75 mL). Triethylamine (12.5 mL) was added, followed by di-*t*-butyldicarbonate (13.08 g, 60 mMol) and ether (75 mL), and the slurry was stirred for 3 h at 25°C. The resulting mixture was diluted with ether and extracted with 1 N aqueous hydrochloric acid. The organic layer was washed with water, dried over magnesium sulfate and the volatiles were removed in vacuo. Recrystallization from 15% dichloromethane-hexane afforded the desired bisprotected diaminopropionic acid as a white powder. ¹H NMR (300MHz) δ 1.46 (9H), 3.64 (2H), 4.20 (1H), 4.29 (1H), 4.42 (2H), 5.46 (1H), 5.88 (1H), 7.31 (2H), 7.33 (2H), 7.57 (2H), 7.76 (2H); anal. calcd. C:64.78 H:6.15 N:6.57. Found C:64.71 H:6.18 N:6.55

Synthesis of Dimeric Nonapeptides:

Dimeric peptides were assembled on solid phase using standard BOC protocols. Sidechain protection of diaminopropionic acid and Asp/Glu was accomplished using Fmoc and 9-fluorenylmethylester groups respectively. Before deprotection of the terminal Boc group, the 9-Fluorenyl derived sidechain protections were removed by treatment with 50% piperidine in dichloromethane. BOP (2 equivalents) in DMF was added and the mixture was shaken at 25°C for 48 h or until a negative Ninhydrin test was observed. The resulting peptides were further processed, isolated, and characterized in the usual manner (treatment with HF, purification by preparative HPLC, characterization by FAB-MS and amino acid analysis).

FAB - MS analysis:

Fast atom bombardment (FAB) mass spectra were obtained on a VG 70SQ mass spectrometer of EBQQ geometry using a VG 11-250J Data System for data

acquisition. The mass spectrometer was operated at seven kilovolts accelerating potential and a resolution of 1000 (10% valley definition). The FAB gun used in the experiments was Io Tech FAB 11N operating at seven kilovolts potential and one milliamperes current. Xenon was used as the bombardment gas at a pressure of 1×10^{-5} millibars source pressure. The sample of interest was dissolved in glycerol prior to analysis by FAB-MS.

The invention is illustrated, but not limited, by the following examples:

The method of synthesis of each peptide is indicated. All peptides were purified by HPLC. Analytical data and the structure of each Example are shown in the Table.

Example 1: IleAsnProAibTyrAgrLeuArgTyr-NH₂ (SEQ.ID.No:8)

The peptide was synthesized by method A. Analytical data in the Table. Purification by HPLC.

Example 2: IleAsnPro(O-MethylThr)TyrArgLeuArgTyr-NH₂ (SEQ.ID.No:9)

The peptide was synthesized by method A. Analytical data in the Table.

Example 3: IleAsnProAib[O-(2,6-Dichlorobenzyl)Tyr]ArgLeuArgTyr-NH₂
(SEQ.ID.No:10)

Synthesis by method B.

Example 4: IleAsnProIleTyrArgLeuArg[O-(2,6-Dichlorobenzyl)-Tyr]-NH₂
(SEQ.ID.No:11)

Synthesis by method B.

Example 5: IleAsnProIle[O-(2,6-Dichlorobenzyl)Tyr]ArgLeuArgTyr-NH₂
(SEQ.ID.No:12)

Synthesis by method B.

Example 6: Synthesis of IleCysProCysTyrArgLeuArgTyr-NH₂,Cyclic(2,2'),
(4,4')-Disulfide Dimer and
IleCysProCysTyrArgLeuArgTyr-NH₂,Cyclic(2,4'),(4,2')-
Disulfide Dimer (SEQ.ID.No:6)

The reduced peptide IleCysProCysTyrArgLeuArgTyr-NH₂ (SEQ.ID.No:6) was assembled in the peptide synthesizer as described above in Method A, cleaved from the resin with liquid HF and purified by preparative HPLC. This preparation was a single peak on analytical HPLC and eluted at 7.53 min at standard conditions. The peptide also was observed to 100% pure on capillary zone electrophoresis (CZE). The correct structure was confirmed by Fab-MS. (MH⁺=1185.7). This peptide gives a yellow colour with the Ellman reagent, indicating the presence of a -SH moiety in the peptide. Forty milligrams of the peptide were dissolved in 500 ml of 20 mM sodium phosphate buffer (pH 8.5) and a 0.1 M solution of potassium ferricyanide was added in 100 microliter portions until the colour of the peptide solution stayed yellow. The solution was then lyophilized and purified by preparative HPLC to yield 10 milligrams of a solid that consisted of two items on analytical HPLC. The first peak (23% of the solid) eluted at 7.46 min under standard conditions. The second peak (77% of the solid) eluted at 7.79 min. CZE of Peak #1 yielded one peak (100% purity based on CZE). Peak #2 yielded two items on CZE ration (1:2). This preparation does not yield a yellow colour with the Ellman test i.e. no free -SH groups are present. FAB-MS of Peak #2 indicates that the two peptides are the two dimer types each of which contain the same monomers but have the monomers arranged in either the same or opposing orientations respectively (MH⁺=2367.4).

Example 6(a): Synthesis of IleCysProCysTyrArgLeuArgTyr-NH₂,Cyclic
(2,2'),(4,4')-Disulfide Dimer (SEQ.ID.No:6) and
IleCysProCysTyrArgLeu-ArgTyr-NH₂,Cyclic(2,4'),(4,2')-
Disulfide Dimer (SEQ.ID.No:6)

The peptide was assembled by the automated Boc-protection procedure as described above in Method A, on a 0.5 mM scale using the standard sidechain protection groups except for cysteine which was introduced as Boc-Cys(S-Fmoc). After the deprotection of the last Boc- group, the peptide resin was treated with a solution of 50% piperidine in DMF for three hours while constantly saturating the solution with oxygen. Cleavage of the peptide from the resin with liquid HF yielded 200 mg of crude peptide which, after purification by preparative HPLC, gave 50 mg pure product. This material was indistinguishable from Peak #2 obtained in Example 6 as shown by HPLC and FAB-MS. CZE indicated the presence of the same two products as obtained in Peak #2 of Example 6.

Example 7: IleAspProDprTyrArgLeuArgTyr-NH₂, Cyclic(2,4'), (2',4)-Diamide
(SEQ.ID.No:7)

Synthesized by method C.

Example 8: IleDprProAspTyrArgLeuArgTyr-NH₂, Cyclic(2,4'), (2',4)-Diamide
(SEQ.ID.No:5)

Synthesized by Method C.

Example 9: IleGluProDprTyrArgLeuArgTyr-NH₂, Cyclic(2,4'), (2',4)-Diamide
(SEQ.ID.No:4)

Synthesized by Method C.

Example 10: IleAsnProIlePheArgLeuArgTyr-NH₂ (SEQ.ID.No:13)

Synthesized by Method A.

Example 11: IleAsn(3,4-DehydroPro)IleTyrArgLeuArgTyr-NH₂ (SEQ.ID.No:14)

Synthesized by Method A.

Example 12: (IleAsnProCysTyrArgLeuArgTyr-NH₂)₂ (SEQ.ID.No:15)

The monomeric structure was prepared using Method A, FAB-MS (MH⁺-1197.0), capillary zone electrophoresis, and amino acid analysis (Arg: 2.01, Asp: 0.83, Ile: 0.96, Leu: 1.00, Pro: 1.44, Tyr: 1.84) verified this structure. Approximately 100mg (8.4×10^{-5} moles) of the monomer was dissolved in 50 mL of Na₂HPO₄ buffer (pH=8). A 0.1 solution of aqueous K₄Fe(SCN)₆ (10 x 100μL, 1×10^{-4} moles) was added and stirred until the yellow colour no longer dissipated. This material was lyophilized and purified by C18 preparative HPLC as described previously. This dimeric structure was verified by FAB-MS (MH⁺- 2391.6), capillary zone electrophoresis, and amino acid analysis (Arg: 1.98, Asp: 1.14, Ile: 0.87, Leu: 1.03, Pro: 1.00, Tyr: 1.97).

Example 13: (IleCysProIleTyrArgLeuArgTyr-NH₂)₂ (SEQ.ID.No:16)

The monomeric structure was prepared using Method A, FAB-MS (MH⁺-1196.1), capillary zone electrophoresis, and amino acid analysis (Arg: 2.08, Ile: 1.28, Leu: 1.00, Pro: 1.31, Tyr: 1.80) verified this structure. Approximately 100mg (8.4×10^{-5} moles) of the monomer was dissolved in 50mL of Na₂HPO₄ buffer (pH=8). A 0.1M solution of aqueous K₄Fe(SCN)₆ (10 x 100μL, 1×10^{-4} moles) was added and stirred until the yellow colour no longer dissipated. This material was lyophilized and purified by C18 preparative HPLC as described previously. This dimeric structure was verified by FAB-MS (MH⁺-2374.1), capillary zone electrophoresis, and amino acid analysis (Arg: 2.76, Ile: 1.56, Leu: 1.00, Pro: 1.18, Tyr: 1.60).

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(Pim - 2,6-Diaminopimelic Acid L.D)

Into 80mL of 5% Na_2CO_3 and 50mL 1,4-dioxane, (\pm)-2,6-diaminopimelic acid (5g, 26.3 mmol, Aldrich) was dissolved and cooled to 0°C . A solution containing 24.2mL (105.2 mmol, Aldrich) of di-tert-butyl dicarbonate in dioxane was added dropwise. After addition was complete, the mixture was allowed to warm to room temperature and stirred for 16 h. The dioxane was then removed in vacuo, and the aqueous was acidified to pH = 1 with 1 N HCl. A colourless oil was obtained and dissolved in 10mL of ethyl acetate. To this, 10.6mL (52.6 mmol, Aldrich) of dicyclohexylamine was added. The mixture was diluted with 250mL of diethyl ether and cooled overnight. A white solid was filtered and dried to yield 9.6g (48.2%) and used without further purification. NMR dmso (ppm): 1.0-2.0m, 44H; 1.4 s, 18H; 3.5-3.6m, 2H; 6.0-6.1m, 2H.

A sample (0.5 mmoles) of p-methylbenzhydrylamine.HCl resin (obtained as before) (MBHA) was loaded onto an Applied Biosystems Inc. Model 430A Peptide Synthesizer and the following amino acids (1.0 mmole each) were coupled to the MBHA resin using a programme to suit a BOP coupling procedure as described by Dung Le Nguyen et al (ibid): Boc-Tyr(2-Br-CBZ), Boc-Arg(Tos), Boc-Leu, Boc-Arg(Tos), and Boc-Tyr(2-Br-CBZ). The MBHA resin was then deprotected utilizing 50% trifluoroacetic acid (TFA)/CH₂Cl₂. The resin was removed from the ABI-430A and manually shaken for 1 h with 184mg (0.25 mmoles) of Boc(L.D)-Pim-DCHA, 112mg (0.25 mmoles) of BOP reagent, and 25μL (0.25 mmoles) of N-methylimidazole (Aldrich). The resin was once again deprotected with TFA and returned to the ABI-430A, where these remaining amino acids were added: Boc-Pro, Boc-Asn(Xan), and Boc-Ile.

The peptide was cleaved from the resin with HF/1% anisole at 0°C, extracted with 10% aqueous acetic acid and lyophilized. Purification was performed on a Waters Prep-Pak Delta-Pak C18 column eluting the peptide with

acetonitrile from 0.1% TFA/H₂O. HPLC purity was verified (one peak only) using an analytical system identical to the preparative HPLC system described above.

FAB-MS showed the correct peptide at 2342.7 (MH⁺). Amino acid analysis corroborated this finding with the following results: Arg (4.29), Asp (1.43), Ile (1.45), Leu (2.00), Pro (2.08), and Tyr (3.91).

Example 15: IleGluProOrnTyrArgLeuArgTyr-NH₂, Cyclic(2,4'), (2',4)-Diamide
(SEQ.ID.No:2)

Synthesized by Method A. Nocy-Glu(OFm) was purchased by Bachem, Inc. (Torrance, CA). Alpha-boc-delta-fmoc-ornithine was synthesized from alpha-boc-ornithine (obtained from Bachem Bioscience, Inc., Philadelphia, PA) using the same procedure described in Method C for alpha-boc-gamma-fmoc-diaminopropionic acid. ¹H NMR (300MHz) δ 1.45 (9H), 1.47-1.99 (4H), 3.23 (2H), 4.21 (1H), 4.42 (2H), 5.02 (1H), 5.10 (1H), 6.00 (1H), 7.22-7.79 (8H).

Anal. Calcd. C: 66.06, H: 6.65, N: 6.16.

Found C: 65.93, H: 6.69, N: 5.97.

Example 16: IleAspProOrnTyrArgLeuArgTyrNH₂, Cyclic(2,4'), (2',4)-Diamide
(SEQ.ID.No:3)

Synthesized as described in Example 15.

Example 17: TyrIleAsnLeuIleTyrArgLeuArgTyr-NH₂ (SEQ.ID.No:18)

Synthesized by Method A.

Example 18: desNH₂TyrIleAsnLeuIleTyrArgLeuArgTyr-NH₂ (SEQ.ID.No:18)

Synthesized by Method A.

BIOLOGICAL EVALUATION

RADIORECEPTOR BINDING ASSAY

Rat brain membranes were incubated with ^3H -NPY up to equilibrium at 37°C in the presence of several peptides at different concentrations and in a final volume of 1 ml. Non-specific binding was determined by adding 100 nM of cold NPY. At the end of the incubation period the samples were either filtered (GF/C filter), or 0.5 ml of cold incubation buffer was added and the tubes centrifuged at 15,000 g for 10 min. The supernatant was discarded and the pellet or filters resuspended and counted by liquid scintillation. Results were processed using a computer programme that calculated IC_{50} , K_d , B_{max} and Hill coefficient by linear regression. IC_{50} results where available are shown in column (a) of the Table.

INTRACELLULAR CALCIUM MEASUREMENTS

Human erythroleukemic (HEL) cells were loaded with Fura-2 ($1\text{ }\mu\text{M}$ for 1 h), washed and then placed in a cuvette (5×10^5 cells in 2.5 ml) and excited simultaneously at 340 and 380 nm in a SLM spectrofluorometer. Fluorescence changes after the addition of NPY and/or peptide analogs were followed by recording emission at 510 nm. Calcium concentrations can be determined through a computer programme by using the ratio of fluorescence 340/380 nm. The agonistic activity of peptide analogs was tested by recording the fluorescence induced upon stimulation of the cells with 100 nM of the peptide and the antagonistic activity by observing the decrease in fluorescence induced by 5 nM NPY in the presence of the analog at 100 nM. The results where available are indicated in column (c) of the Table.

The inhibition of the increase in the HEL cell intracellular calcium (induced by 5nM NPY) achieved by 100nM concentrations of each compound is shown in column (b) of the Table.

ISOLATED PERFUSED RAT KIDNEY PREPARATION

Male CD rats (200-400 g) are anaesthetised with a mixture of Acepromazine (2.8mg/kg) and Ketamine (112mg/kg) injected i.p.. The rat's abdomen is opened through the midline. The renal arteries and descending aorta are cleaned and, with minimal disruption of blood flow, one kidney is cannulated through the proximal renal artery using a stainless steel 20G blunt-tipped needle attached to Tygon tubing and a peristaltic pump. As soon as the first ligature is complete and perfusion of the kidney starts (10ml/min) and the kidney is removed from the animal and placed in a constant temperature perfusion chamber. The perfusion pressure is followed by an on-line Statham pressure transducer and changes recorded in a Grass Polygraph.

Administration of Drugs: Neuropeptide Y and other agonists are administered by a single bolus injection (0.1ml) through an injection port situated about 6 inches from the tip of the needle (approximately 0.3ml, total volume). Antagonists are infused during 5 min (0.15ml/min) into the kidney through an injection port situated before the perfusion pump to allow for good mixing and constant concentration (dilution of 0.15ml into 10ml).

Perfusion Buffer (mM): NaCl 122; KCl 4.73; NaHCO₃ 15.5; KH₂PO₄ 1.19; CaCl₂ 2.5; MgCl₂ 1.19; Glucose 11.5 and 1% BSA pH 7.4. The buffer is constantly bubbled with 95% O₂, 5% CO₂.

Results: Using the above experimental procedure we have determined the IC₅₀ for the inhibition of the NPY-induced increase of perfusion pressure for:

IC₅₀(nM)

IleCysProCysTyrArgLeuArgTyr-NH ₂	
Cyclic (2,2'),(4,4')-Disulfide Dimer (SEQ.ID.No:6)	2.5
and	
IleCysProCysTyrArgLeuArgTyr-NH ₂	
Cyclic (2,4'),(4,2')-Disulfide Dimer (SEQ.ID.No:6)	2.5
 IleGluProDprTyrArgLeuArgTyr-NH ₂	
Cyclic (2,4'),(2',4)-Diamide (SEQ.ID.No:4)	0.5

TABLE

HEL CELLS

DRAIN

EXAMPLE

NO.

STRUCTURE

IC₅₀ (m) 100% Inhib 1 Fluor IC₅₀ (m) MH⁺ (FAD)-MS
(a) (b) (c) (d)

AAA

1.	IleAsnProAlbTyrArg LeuArgTyr-MH ₂ (SEQ. ID. No:8)	0.25	100	0	0.010	1170.9	Arg(1.79), Asp(0.69), Ile(0.70), Leu(1.00), Pro(0.73), Tyr(1.81)
2.	IleAsnPro(O-MethylThr) TyrArgLeuArgTyr-MH ₂ (SEQ. ID. No:9)	0.28	100	0	0.005	1200.6	Arg(1.80), Asp(0.90), Ile(0.92), Leu(1.00), Pro(1.02), Tyr(1.75)
3.	IleAsnProAlb[O-(2,6-Dichlorobenzyl)]TyrArg LeuArgTyr-MH ₂ (SEQ. ID. No:10)	0.35	100	0	0.008	1337.4	Arg(2.04), Asp(0.09), Ile(0.16), Leu(1.00), Pro(0.19), Tyr(1.91)
4.	IleAsnProIleTyrArg LeuArg[O-(2,6-Dichlorobenzyl)]-Tyr-MH ₂ (SEQ. ID. No:11)	1.4	100	0	0.010	1366.8	Arg(1.94), Asp(0.29), Ile(1.19), Leu(1.00), Pro(1.03), Tyr(1.60)

HPLC DATA

EXAMPLE NO.	STRUCTURE	HPLC DATA					MS
		IC ₅₀ (m) (a)	100% (b)	100% (c)	IC ₅₀ (m) (d)	MW ¹ (FAB) -MS	
5.	IleAsnProIle(O-(2,6-Dichlorobenzyl)Tyr)ArgLeuArgTyr-NH ₂ (SEQ.ID.No:12)	0.05	100	0	0.0025	1365.9	Arg(2.02), Asp(0.80), Ile(1.03), Leu(1.00), Pro(0.40), Tyr(1.94)
6.	IleCysProCysTyrArg LeuArgTyr-NH ₂ Cyclo (2,2'), (4,4')- Disulfide Dimer and IleCysProCysTyrArg LeuArgTyr-NH ₂ Cyclo (2,4'), (4,2')- Disulfide Dimer (SEQ.ID.No:16)	0.25	100	0	0.002	2367.5	Arg(3.80), Ile(1.20), Leu(2.00), Pro(2.12), Tyr(3.60)
7.	IleAspProDprTyrArgLeu ArgTyr-NH ₂ Cyclo (2,4'), (2',4')- Disulfide (SEQ.ID.No:17)	0.015	100	0	0.0015	1162.24 (2324.0)	Arg(1.87), Asp(0.02), Ile(0.81), Leu(1.00), Pro(1.24), Tyr(1.91)

BRAIN HEL. CELLS

EXAMPLE

NO.	STRUCTURE	IC ₅₀ (m)				Fluor		IC ₅₀ (m)		MH ⁺ (FAB) - MS		AAA
		(a)	(b)	(c)	(d)	(e)	(f)	(g)	(h)	(i)	(j)	
12.	(IleAsnProCysTyrArg LeuArgTyr-NH ₂) ₂ (SEQ. ID. No:15)	0.005	100	0	0.0005	2191.6						Arg(3.97), Asp(2.20), Ile(1.75), Pro(2.00), Tyr(3.94), Leu(0.07) (value for dimer)
13.	(IleCysProIleTyrArg LeuArgTyr-NH ₂) ₂ (SEQ. ID. No:16)	0.035	100	0	0.00075	2174						Arg(3.52), Ile(3.13), Leu(2.00), Pro(2.36), Tyr(3.21) (value for dimer)
14.	IleAsnPro TyrArgLeuArgTyr-NH ₂ P1m IleAsnPro TyrArgLeuArgTyr-NH ₂ (SEQ. ID. No:17)	0.0019	100	0	0.005	2342.7						Arg(4.29), Asp(1.43), Ile(1.45), Leu(2.00), Pro(2.00), Tyr(3.9)

(P1m = 2,6- Diaminopimelic acid 1,0)

REL. CELLS

EXAMPLE

NO.	STRUCTURE	REL. CELLS			
		IC ₅₀ (m) (a)	% INHIB (b)	% FLUOR (c)	IC ₅₀ (m) (d)

AAA

MI¹ (FAD)-MS

18. deaIII₂TyrIleLeuLeuIle
TyrArgLeuArgTyr-MII₂
(SEQ. ID. No: 18)

0.017

62

20

II/A

1371.7

Arg(2.20), Asp(0.79),
Ile(1.93), Leu(2.00),
Tyr(1.96)

HET. CELLS

BRAIN

EXAMPLE

NO.	STRUCTURE	IC ₅₀ (m)				Fluor		IR ¹ (FAB) -MS		AAA
		(a)	(b)	(c)	(d)	(a)	(b)	(c)	(d)	
15.	IleGluProOrnTyrArgLeu ArgTyr-NH ₂ Cyclic (2,4'), (2',4')- Diamide (SEQ.ID.No:2)	<0.0001	100	0	0.00025	1295.24 (2400.0)				Arg(2.07), Glx(0.96), Ile(0.93), Leu(1.00), Pro(1.06), Tyr(1.67)
16.	IleAspProOrnTyrArgLeu ArgTyr-NH ₂ Cyclic (2,4'), (2',4')- Diamide (SEQ.ID.No:3)	<0.0001	100	0	0.00025	1191.24 (2300.8)				Arg(2.09), Asx(0.56), Ile(0.58), Leu(1.00), Pro(1.36), Tyr(2.17)
17.	TyrIleAsnLeuIleTyrArg LeuArgTyr-NH ₂ (SEQ.ID.No:8)	0.015	100	10	0.00075	1306.3				Arg(1.96), Asx(1.09), Ile(1.39), Leu(2.00), Tyr(2.64)

HPLC CELLS

EXAMPLE

NO.	STRUCTURE	HPLC CELLS				AAA
		IC ₅₀ (m) (a)	IC ₅₀ (m) (b)	Fluor (c)	IC ₅₀ (m) (d)	
8.	IleDprProAspTyrArg LeuArgTyr-NH ₂ Cyclic (2,4'), (2',4')- Diamide (SEQ.ID.No:5)	0.0075	100	0	0.0025 1162.54 (2324.8)	Arg(1.90), Asp(1.09), Ile(0.29), Leu(1.00), Pro(1.23), Tyr(2.05)
9.	IleGluProDprTyrArg LeuArgTyr-NH ₂ Cyclic (2,4'), (2',4')- Diamide (SEQ.ID.No:4)	0.001	100	0	0.00029 2353.9	Arg(1.89), Glu(0.81), Ile(0.83), Leu(1.00), Pro(1.18), Tyr(1.97)
10.	IleAsnProIlePheArg LeuArgTyr-NH ₂ (SEQ.ID.No:13)	0.47	100	0	0.01 1191.0	Arg(2.00), Asp(0.91), Ile(1.98), Leu(1.05), Phe(0.96), Pro(1.06), Tyr(1.00)
11.	IleAsn(3,4-Dihydro- Pro)IleTyrArgLeuArg Tyr-NH ₂ (SEQ.ID.No:14)	0.036	100	0	0.01 1205.0	Arg(1.85), Asp(0.90), Ile(1.66), Leu(1.00), Tyr(1.66)

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(E) COUNTRY: United States
(F) POSTAL CODE (ZIP): 27606

(ii) TITLE OF INVENTION: PEPTIDES

(iii) NUMBER OF SEQUENCES: 18

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: /note= "Xaa is Ile, Ile Tyr, or
desNH2TyrIle"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2
- (D) OTHER INFORMATION: /note= "Xaa is Asn, Asp, Cys, Dpr,
Glu, or Gly"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3
- (D) OTHER INFORMATION: /note= "Xaa is Leu, Pro, or
3,4-dehydroPro"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 4
- (D) OTHER INFORMATION: /note= "Xaa is Aib, Asp, Cys,
Dpr, Glu, Gly, Ile, Orn, Tyr, or O-methylThr"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: /note= "Xaa is Phe, Tyr, or
O-(2,6-dichlorobenzyl)Tyr"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 7
- (D) OTHER INFORMATION: /note= "Xaa is Phe or Leu"

(2

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(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 9
- (D) OTHER INFORMATION: /note= "Xaa is Phe, Tyr, or
O-(2,6-dichlorobenzyl)Tyr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Xaa Xaa Xaa Xaa Xaa Arg Xaa Arg Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 4
- (D) OTHER INFORMATION: /note= "Xaa is Orn"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Ile Glu Pro Xaa Tyr Arg Leu Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(2:

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note- "Xaa is Orn"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Ile Asp Pro Xaa Tyr Arg Leu Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(2)

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
(B) LOCATION: 4
(D) OTHER INFORMATION: /note- "Xaa is Dpr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ile Glu Pro Xaa Tyr Arg Leu Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2
- (D) OTHER INFORMATION: /note= "Xaa is Dpr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ile Xaa Pro Asp Tyr Arg Leu Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Ile Cys Pro Cys Tyr Arg Leu Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(2

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 4
- (D) OTHER INFORMATION: /note= "Xaa is Dpr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Ile Asp Pro Xaa Tyr Arg Leu Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids

- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 4
- (D) OTHER INFORMATION: /note= "Xaa is Aib"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Ile Asn Pro Xaa Tyr Arg Leu Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 4
- (D) OTHER INFORMATION: /note= "Xaa is O-methylThr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Ile Asn Pro Xaa Tyr Arg Leu Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 4
- (D) OTHER INFORMATION: /note= "Xaa is Aib"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: /note= "Xaa is
O-(2,6-dichlorobenzyl)Tyr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Ile Asn Pro Xaa Xaa Arg Leu Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 9
- (D) OTHER INFORMATION: /note= "Xaa is
O-(2,6-dichlorobenzyl)Tyr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Ile Asn Pro Ile Tyr Arg Leu Arg Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5

(D) OTHER INFORMATION: /note- "Xaa is
O-(2,6-dichlorobenzyl)Tyr"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Ile Asn Pro Ile Xaa Arg Leu Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(2

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Ile Asn Pro Ile Phe Arg Leu Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(2

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 3
- (D) OTHER INFORMATION: /note- "Xaa is 3,4-dehydroPro"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Ile Asn Xaa Ile Tyr Arg Leu Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Ile Asn Pro Cys Tyr Arg Leu Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Ile Cys Pro Ile Tyr Arg Leu Arg Tyr
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(2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 4
- (D) OTHER INFORMATION: /note= "Xaa is
2,6-diaminopimelic acid L,D"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

Ile Asn Pro Xaa Tyr Arg Leu Arg Tyr
1 5

(2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Tyr Ile Asn Leu Ile Tyr Arg Leu Arg Tyr

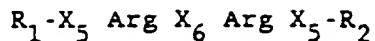
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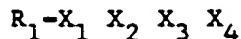
CLAIMS

1. A peptide of formula (I), or a multimer thereof or a salt thereof:



(I) (SEQ.ID.No:1)

where



in which X_1 -Ile, IleTyr, or IledesNH₂Tyr

X_2 -Asn, Asp, Cys, Dpr, Glu or Gly

X_3 -Leu, Pro or 3,4-DehydroPro

X_4 -Aib, Asp, Cys, Dpr, Glu, Gly, Ile, Orn, Tyr or O-MethylThr

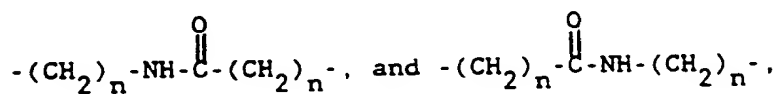
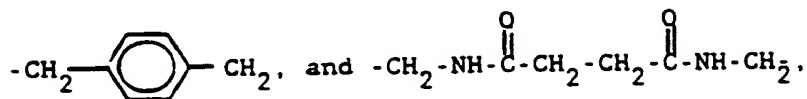
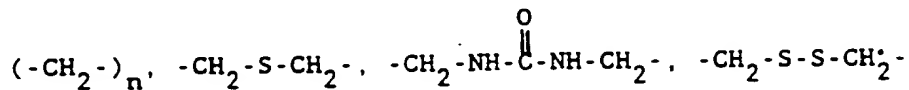
X_5 -Phe, Tyr or [O-(2,6-Dichlorobenzyl)Tyr]

X_6 -Phe or Leu

where R_2 --(CH₂)_nCH₃, --(CH₂)_nNH₂ or $-\overset{\text{O}}{\underset{\text{||}}{\text{C}}}-\text{CH}_3$ in which n=0-4.

2. A peptide according to claim 1, wherein X_2 is selected from Dpr, Cys, Asp and Glu.
3. A peptide according to claim 1 or 2, wherein X_3 is Pro.
4. A peptide according to any of claims 1 to 3, wherein X_4 is selected from Asp, Cys, Dpr and Orn.
5. A peptide according to any of claims 1 to 4, wherein X_5 is Tyr.
6. A peptide according to any of claims 1 to 5, wherein R_2 is --(CH₂)_nNH₂.
7. A multimer of a peptide according to any of claims 1 to 6.

8. A multimer according to claim 7, wherein the multimer is a dimer or trimer.
9. A multimer according to claim 7 or 8, wherein the peptides containing Gly residues are bridged at the alpha positions to form one or more bridges, by one or more groups selected from



wherein $n=0-4$.

10. A peptide, multimer thereof or salt thereof, the peptide having a structure selected from:

IleGluProOrnTyrArgLeuArgTyr-NH₂, Cyclic(2,4'), (2',4)-Diamide
(SEQ.ID.No:2),

IleAspProOrnTyrArgLeuArgTyr-NH₂, Cyclic(2,4'), (2',4)-Diamide
(SEQ.ID.No:3),

IleGluProDprTyrArgLeuArgTyr-NH₂, Cyclic(2,4'), (2',4)-Diamide
(SEQ.ID.No:4),

and

IleDprProAspTyrArgLeuArgTyr-NH₂, Cyclic(2,4'), (2',4)-Diamide
(SEQ.ID.No:5).

11. A peptide, multimer thereof or salt thereof, the peptide having a structure selected from:

IleCysProCysTyrArgLeuArgTyr-NH₂,Cyclic(2,2'),(4,4')-Disulfide Dimer
(SEQ.ID.No:6),

and

IleCysProCysTyrArgLeuArgTyr-NH₂,Cyclic(2,4'),(4,2')-Disulfide Dimer
(SEQ.ID.No:6).

12. A peptide, multimer thereof or salt thereof, the peptide having the structure:

IleGluProDprTyrArgLeuArgTyr-NH₂,Cyclic(2,4'),(2',4)-Diamide
(SEQ.ID.No:4).

13. A peptide, multimer thereof or salt thereof, the peptide having the structure:

IleDprProAspTyrArgLeuArgTyr-NH₂,Cyclic(2,4'),(2',4)-Diamide
(SEQ.ID.No:5).

14. A pharmaceutically acceptable salt of a peptide or multimer according to any of claims 1 to 13.

15. A peptide or multimer according to any of claims 1 to 13, or a pharmaceutically acceptable salt thereof, for use in the medical treatment of a mammal, such as a human being.

16. A peptide or multimer according to any of claims 1 to 13, or a pharmaceutically acceptable salt thereof, for use in the treatment of cardiovascular disorders, hypertension, hypertensive crisis,

vasospasm, cerebral or coronary vasospasm, eating disorders, depression and glaucoma in a human being.

17. A peptide or multimer according to any of claims 1 to 13, or a pharmaceutically acceptable salt thereof, for use in the treatment of stroke.
18. Use of a peptide or multimer according to any of claims 1 to 13, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for the medical treatment of a mammal such as a human being.
19. Use of a peptide or multimer according to any of claims 1 to 13, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for the treatment of cardiovascular disorders, hypertension, hypertensive crisis, vasospasm, cerebral or coronary vasospasm, eating disorders, depression and glaucoma in a mammal such as a human being.
20. Use of a peptide or multimer according to any of claims 1 to 13, or a pharmaceutically acceptable salt thereof for the manufacture of a medicament for the treatment of stroke in a mammal such as a human being.
21. A pharmaceutical formulation comprising a peptide or multimer according to any of claims 1 to 13, or a pharmaceutically acceptable salt thereof, together with an acceptable carrier therefor.
22. A method for the treatment of cardiovascular disorders, hypertension, hypertensive crisis, vasospasm, cerebral or coronary vasospasm, eating disorders, depression and glaucoma, which requires administering to a human being, a therapeutically effective, non-toxic amount of a peptide or multimer according to any of claims 1 to 13.

23. A method for the treatment of stroke which requires administering to a human being, a therapeutically effective, non-toxic amount of a peptide or multimer according to any of claims 1-13.

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I. CLASSIFICATION OF SUBJECT MATTER

(If several classification symbols apply, indicate all)⁶

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C07K7/06;

A61K37/02

II. FIELDS SEARCHED

Minimum Documentation Searched⁷

Classification System

Classification Symbols

Int.Cl. 5

C07K

Documentation Searched other than Minimum Documentation
to the extent that such documents are included in the fields searched⁸III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹

Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A	EP,A,0 355 794 (MERRELL DOW PHARMACEUTICALS INC.) 28 February 1990 cited in the application see the whole document ---	1-23
A	J.A.SMITH 'PEPTIDES, Chemistry and Biology, Proc. 12th A.P.S., June 16-21, 1991, Cambridge, U.S.A.' 1992, ESCOM, LEIDEN A.G.Beck-Sickinger et al; "Structure-activity relationships of NPY" see page 17 - page 19 --- -/--	1-23

¹⁰ Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"A" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

14 SEPTEMBER 1993

Date of Mailing of this International Search Report

29 -09- 1993

International Searching Authority

EUROPEAN PATENT OFFICE

Signature of Authorized Officer

GROENENDIJK M.S.M.

III. DOCUMENTS CONSIDERED TO BE RELEVANT

(CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	<p>J.A.SMITH 'Peptides, Chemistry and Biology, Proc. 12th A.P.S., June 16-21, 1991, Cambridge, U.S.A.'</p> <p>1992, ESCOM, LEIDEN</p> <p>D.A.Kirby et al; "Structure-activity relationships of conformationally restricted deletion analogs of NPY" see page 480 - page 481</p> <p>-----</p>	1-23

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos. because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 22, 23 are directed to a method of treatment of (dia-nostic method practised on) the human/animal body the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos. because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos. because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

GB 9301297
SA 75344

14/09/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0355794	28-02-90	AU-B- 620432	20-02-92
		AU-A- 4017689	01-03-90
		JP-A- 2111793	24-04-90

EPO FORM 1001

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82